# Indirect evidence of nasal inflammation assessed by titration of inflammatory mediators and enumeration of cells in nasal secretions of patients with chronic rhinitis

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*Pathophysiologic mechanisms of perennial rhinitis are poorly understood. The characterization of inflammation was studied in nasal lavage of patients with perennial rhinitis by the enumeration of cells involved in the allergic inflammation and the measurement of six mediators released in nasal secretions to determine whether some mediators were relevant for the etiologic diagnosis and the occurrence of symptoms. Ten healthy subjects and 57 patients with perennial rhinitis were placed into four groups according to the symptoms they presented at the time of the study and the origin of the allergy. Allergy was characterized by the history, skin prick tests to standardized allergens, and RAST. Eosinophil protein X (EPX), tryptase, histamine, myeloperoxidase, prostaglandin $D_2$, and leukotriene $C_4/D_4$ ($LTC_4/D_4$) were measured in nasal lavage by enzyme assay or radioimmunoassay. Eosinophils and neutrophils were enumerated after cytocentrifugation of the lavage fluid and May Grunwald Giemsa staining. Tryptase, myeloperoxidase and EPX but not histamine levels were increased in all four patient groups. Eosinophils, $LTC_4/D_4$, and prostaglandin $D_2$ were significantly ($p < 0.001$, $p < 0.03$, and $p < 0.01$) increased in allergic and symptomatic patients. EPX was significantly increased in symptomatic allergic and nonallergic patients. This study suggests the involvement of mast cells, neutrophils, and eosinophils, but the latter cells appear to have a more prominent role. The importance of EPX and $LTC_4/D_4$ in the characterization of chronic symptomatic rhinitis was also observed. (J ALLERGY CLIN IMMUNOL 1992;90:880-9.)*

***Key words:*** *Rhinitis, inflammation, allergy, eosinophils, neutrophils, histamine, tryptase, $PGD_2$, $LTC_4$*

The pathophysiologic mechanisms underlying nasal allergic diseases have largely been studied in pollen allergy.[1, 2] The studies of mediator release after nasal allergen challenge pioneered by Naclerio et al.[3] made it possible to analyze the events of the allergic reaction more precisely. Cells and mediators occurring during the early- and late-phase reactions,[4, 5] as well as during rechallenge,[6] have shown that the allergic inflammation caused by the coupling of mast cell–bound IgE to specific allergens leads to the release of vasoactive (histamine,[3, 6-8] sulfidopeptide leukotrienes,[8-12] prostaglandin $D_2$ [$PGD_2$][3, 6, 7] or kinins[3, 6, 13]) and chemotactic mediators,[9] tryptase,[14, 15] eosinophil-derived

*Abbreviations used*

| | |
|---|---|
| A S+: | Allergic and symptomatic |
| A S−: | Allergic and symptom-free |
| NA S+: | Nonallergic symptomatic |
| NA S−: | Nonallergic symptom-free |
| ECP: | Eosinophil cationic protein |
| EPX: | Eosinophil protein X (eosinophil-derived neurotoxin) |
| $LTC_4/D_4$: | Leukotriene $C_4/D_4$ |
| NARES: | Nonallergic rhinitis with eosinophils syndrome |
| $PGD_2$: | Prostaglandin $D_2$ |

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granule proteins,[16-18] and the mucosal infiltration by metachromatic cells,[20, 21] eosinophils,[21-24] and possibly neutrophils.[16] However, nasal challenge does not completely represent the natural pollen exposure, and several studies that have examined the occurrence of mediators and cells in nasal secretions collected during the pollen season have often, but not always confirmed the activation of mast cells and eosinophils.[15, 21-35]

Perennial rhinitis is a more complex disease, including allergic rhinitis, the nonallergic rhinitis with eosinophilia syndrome (NARES) and the loosely defined vasomotor rhinitis.[36-39] Symptoms caused by allergic rhinitis or the NARES are similar to rhinorrhea, nasal obstruction, sneezing, and pruritus. On the other hand, in patients with vasomotor rhinitis the major symptom is nasal obstruction.[40] Nasal inflammation has only been examined in few studies, and it has been observed that patients have an inconstant nasal eosinophilia even in allergic rhinitis.[35, 41] The release of mediators in nasal secretions has been examined in one study, and histamine was not found to be increased.[42]

Some of the cells involved in the allergic inflammation and release of specific mediators include eosinophils that release granule constituents such as major basic protein and eosinophil cationic protein (ECP) and eosinophil protein X (EPX),[43-45] mast cells releasing histamine and tryptase,[46-49] and neutrophils releasing myeloperoxidase, which is stored in the azurophil granule and released during phagocytosis or cell activation.[50] On the other hand, sulfidopeptide leukotrienes and $PGD_2$ are released by a variety of cells, including mast cells.[51, 52]

The characterization of inflammation was studied in nasal lavage of patients suffering from perennial rhinitis by the enumeration of cells involved in the allergic inflammation and the measurement of mediators released in nasal secretions. Six mediators were selected because of their relevance to studies of nasal challenge or their importance during the pollen season: EPX, tryptase, histamine, myeloperoxidase, $PGD_2$, and sulfidopeptide leukotrienes. Four groups of patients with perennial rhinitis were studied according to the symptoms they presented at the time of the study and the origin of the allergy to determine whether some mediators were relevant for the etiologic diagnosis and the occurrence of symptoms. The major goal of the study was the measurement of mediators in nasal secretions, and the second goal was the enumeration of cells. This is why we deliberately used nasal lavage to recover cells rather than an alternative method such as the brush method,[53] which might modify the release of mediators.

**TABLE I.** Symptom score of chronic rhinitis

| | |
|---|---|
| Sneezing | |
| Occasional | 1 |
| Common | 2 |
| Common and usually >5 | 3 |
| Rhinorrhea | |
| Anterior | 1 |
| Posterior | 1 |
| Both symptoms | 3 |
| Blockade | |
| Patient can breathe freely | 0 |
| Patient can only breathe with difficulty | 1 |
| One nostril is blocked | 2 |
| Both nostrils are blocked | 3 |
| Pruritus | 1 |

## MATERIAL AND METHODS

### Subjects

Fifty-seven patients (26 men, age: 31.3 ± 16.4 years) volunteered to participate in the study after informed consent was given. All had symptoms of perennial rhinitis characterized by anterior rhinorrhea and nasal obstruction, inconstant sneezing, and nasal pruritus. Eighteen also had symptoms of conjunctivitis. The duration of symptoms had ranged from 2 to 30 years. Thirty-three patients had current symptoms, and 24 had had symptoms between 1 to 10 days before the study. None of the patients had received any form of specific immunotherapy or had acute or chronic sinusitis.

Ten healthy volunteers (22 to 45 years old; mean ± SD, 30.0 ± 4.1 years) were used as a control group. They were nonallergic and had never suffered from seasonal or perennial rhinitis.

It was important that none of the subjects had had any nasal infection within the previous month, since histamine and other mediators may be elevated in nasal secretions.[54] Patients were excluded from the study if they had taken systemic corticosteroids of any form during the past 2 months, topical corticosteroids the previous month, sodium cromoglycate, $H_1$-blockers, or ketotifen the week before the test. None of the patients had been treated with astemizole.

The study was conducted after informed consent had been obtained from the participants and after approval of the study by the ethics committee of the hospital.

### Investigations

*Rhinitis score.* The clinical severity of rhinitis was quoted according to a symptom score previously used in seasonal rhinitis (Table I).[55] The clinical score of rhinitis was filled in by a single investigator (J.K.) who performed the whole clinical study.

*Etiologic investigations of rhinitis.* All patients underwent identical investigations. Allergy tests, including a battery of extracts of common food and aeroallergens found

in the Montpellier area,[56] were skin prick tests performed according to a technique previously described in detail elsewhere.[57] Total serum IgE (Phadebas PRIST; Pharmacia Diagnostics AB, Uppsala, Sweden) and Phadiatop (Pharmacia) completed the study. Serum-specific IgE was titrated by the Phadebas RAST (Pharmacia) in patients with positive skin prick tests. All patients had sinus radiography.

*Nasal washing.* A wash with 5 ml of saline solution in each nostril was performed. The wash fluid was immediately centrifuged at +4° C for 15 minutes at 15,000*g*, and the sol phase was separated from the gel phase by use of a pipette and stored at −20° C until assay was performed.

*Examination of nasal lavage cells.* The nasal cytology was performed on cytocentrifuged preparations (Shandon, U.K.) stained with use of May Grundwald Giemsa according to the method of Pipkorn and Karlsson.[58] Cells were only enumerated when at least 50 cells could be counted.

*Titration of mediators in nasal washing.* ECP was not titrated, because in a pilot study we observed that when we used a double antibody radioimmunoassay (Pharmacia), its levels were not often increased in nasal lavage fluids of patients with allergenic perennial rhinitis. Instead, we used EPX as a marker of eosinophils. It was titrated by means of a double-antibody radioimmunoassay (Pharmacia) with a polyclonal rabbit antibody as previously described by Carlson et al.[59] EPX in samples competes with a fixed amount of iodine 125 ($^{125}$I)–labeled EPX for the binding sites of specific antibodies. The EPX standards are calibrated against pure EPX prepared according to Peterson and Venge.[60] Levels under 0.7 μg/L are undetectable. The interassay coefficient of variation was under 10%. Cross-reactivity of the assay with ECP from eosinophils was <0.03%.

Tryptase was titrated in unconcentrated nasal lavage with use of a commercially available kit (Pharmacia). In brief, the assay is a solid-phase radioimmunoassay based on two tryptase-specific monoclonal antibodies.[61, 62] In the assay the tryptase in the sample reacts with antitryptase antibody bound to the wall of the test tube. Then $^{125}$I-antitryptase is added to form a labeled complex so that tryptase in the sample reacts simultaneously with the solid phase antitryptase bound to the test tube and $^{125}$I-antitryptase forming an antitryptase-tryptase $^{125}$I-antitryptase complex. After an overnight incubation at room temperature, the tubes were washed three times, and the remaining radioactivity was determined. The intraassay and interassay coefficients of variation are less than 4% for samples. Levels of tryptase under 0.5 μg/L are undetectable,[63] and the tryptase standards are calibrated against pure tryptase prepared with use of the method of Schwartz et al.[64]

Myeloperoxidase was titrated with use of a double antibody radioimmunoassay (Pharmacia). Myeloperoxidase in samples competes with a fixed amount of $^{125}$I-labeled myeloperoxidase for the binding sites of a specific polyclonal rabbit antibody. The technique used was as stated on the package insert. The interassay coefficient of variation was under 12%, and levels under 8 μg/L are undetectable.

Histamine was titrated by radioimmunoassay with a monoclonal antibody against acylated-histamine (Immunotech, Marseille, France),[65] and the limit of detectability is 0.05 ng/ml. Since in some studies the levels of histamine were not found to be increased after nasal challenge with allergen or in studies performed in seasonal or perennial rhinitis,[7, 8, 23, 25, 42] it is possible that histamine might have been degraded into methylhistamine, which is not recognized by the monoclonal antibody against histamine used in the present study. We therefore verified the validity of our histamine assay in 30 samples by comparing the titration of histamine using the Immunotech technique with that of methylhistamine and histamine by use of a commercially available kit (Pharmacia), and we observed a strong correlation between both assays ($r_s = 0.85, p < 0.001$, Spearman rank test). This experiment is consistent with previous comparative studies and confirms the validity of the assay used in the present study.[66, 67]

$PGD_2$ was assayed by enzyme immunoassay according to the method of Maclouf et al.,[68] and acetylcholinesterase from electric eels was the enzyme used in the assay.[69] The technique used, which is commercially available (Stallergènes Laboratories, Fresnes, France), was described in detail in a previous article.[7] The limit of detectability is 30 pg/ml.

Leukotriene $C_4/D_4$ ($LTC_4/D_4$) was assayed by enzyme immunoassay with use of a commercially available kit (Stallergènes Laboratories), and acetylcholinesterase from electric eels was the enzyme used in the assay.[70] The antibody used in this assay shows a cross-reactivity at 50% $B/B_0$ of 46% with $LTD_4$ and 2% with $LTE_4$ at +22° C. The limit of detectability is 15 pg/ml.

## Design of the study

*Classification of patients.* Patients were classified as "allergic" if they had (1) a history suggestive of perennial rhinitis throughout the year with an exacerbation during the late summer and autumn, when mite and mold counts are at the higest in our area, or if they had symptoms for more than 4 months during the late summer and autumn, and (2) positive skin tests and RAST to house dust mites and/or molds. Patients were classified as "nonallergic" if they had (1) a history suggestive of perennial rhinitis for over 4 months without any exacerbation during the late summer and autumn, (2) no positive skin test to perennial allergens including house dust mites and molds, and (3) a negative Phadiatop outcome. Only patients with these clear-cut characteristics were entered in the study. Patients were classified into four groups according to the origin of allergy and the symptoms they presented on the day of the study: allergic rhinitis and current symptoms (A S+), allergic rhinitis without current symptoms (A S−), nonallergic rhinitis with current symptoms (NA S+), and nonallergic rhinitis without current symptoms (NA S−). The study was carried out between October and December, at a time when pollens are found in insignificant amounts in the Montpellier area.

*Statistical analysis.* Statistical analyses were carried out by means of nonparametric tests. The Kruskal-Wallis test was used to compare all groups, and the Mann-Whitney U test was used for individual group comparisons. Spearman

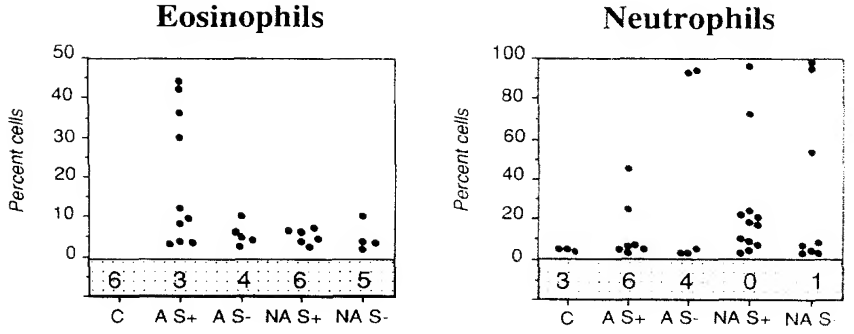


**FIG. 1.** Cells recovered by nasal lavage. Only patients with enumerated slides are presented. Numbers indicate patients with undetectable eosinophils or neutrophils.

**TABLE II.** Global results

| | | A S+ | A S− | NA S+ | NA S− | Control |
|---|---|---|---|---|---|---|
| No. of subjects | | 19 | 13 | 14 | 11 | 10 |
| Age (yr) | | 23.2 ± 12.5 | 24.6 ± 11.8 | 38.4 ± 13.6 | 44.5 ± 20.2 | 30.0 ± 4.1 |
| Symptoms | | | | | | |
| Rhinorrhea | m ± SD | 2.1 ± 0.9 | 1.9 ± 0.3 | 1.7 ± 0.5 | 2.4 ± 0.5 | 0 |
| | % patients | 100 | 100 | 100 | 100 | |
| Obstruction | m ± SD | 1.7 ± 0.5 | 1.6 ± 0.7 | 1.6 ± 0.6 | 1.2 ± 0.5 | 0 |
| | % patients | 100 | 100 | 100 | 91 | |
| Pruritus | m ± SD | 0.6 ± 0.6 | 0.7 ± 0.5 | 1.0 ± 0.8 | 0.4 ± 0.5 | 0 |
| | % patients | 58 | 77 | 86 | 100 | |
| Sneezing | m ± SD | 1.8 ± 1.0 (a) | 0.6 ± 0.8 (b) | 0.7 ± 0.6 (c) | 0.3 ± 0.5 | 0 |
| | % patients | 90 | 54 | 64 | 46 | |
| Conjunctivitis | % patients | 47% | 73% | 0 | 0 | 0 |
| % Patients with cells in nasal lavage | | | | | | |
| Cells enumerated | | 68.4 | 69.2 | 85.7 | 81.9 | 60 |
| Eosinophils* | | 82.1 | 55.6 | 33.3 | 44.4 | 0 |
| Neutrophils* | | 53.8 | 55.6 | 100 | 81.3 | 50 |

$p$ a/b $< 0.01$, $p$ a/c $< 0.01$ (Mann-Whitney U test).
*Percentage in patients with enumerated cells.

rank correlations were not performed when the levels of a mediator were undetectable in more than 50% of samples. Results are given in mean or median ± standard deviations.

## RESULTS

### Characteristics of the patients

Mean ages of patients with rhinitis and healthy subjects were not significantly different. However, patients with allergic rhinitis (A S+ and A S−) were significantly younger ($p < 0.005$, Mann-Whitney U test) than those with nonallergic rhinitis (NA S+ and NA S−) (Table II). All allergic patients had a house dust mite allergy assessed by positive skin tests and RASTs. Other sensitivities included molds (6 patients), animal danders (7 patients), and 12 patients had both perennial and seasonal symptoms as a result of pollen allergy.

Five patients in the A S− group and two patients in the A S+ group had symptoms that subsided during the summer. All nasal symptoms except sneezing were equally distributed among the four groups of patients (Table II). In particular, obstruction was not more severe in the nonallergic group. Sneezing was significantly more severe in A S+ patients than in A S− or in NA S+ patients ($p < 0.01$, Mann-Whitney U test). Only patients in the allergic group (A S+ and A S−) had conjunctivitis.

### Nasal lavage cells

A readable cytocentrifuge slide was obtained on 68.2% to 85.7% of patients and 60% of control subjects. Eosinophils were only found in patients with rhinitis. In the A S+ group eosinophils were enumerated in 82.1% of readable slides. In the other three

**TABLE III.** Statistical evaluation of the data

| | Control subjects vs patients with rhinitis | | | | Allergic vs nonallergic | |
|---|---|---|---|---|---|---|
| | A S+ | A S− | NA S+ | NA S− | A S+/NA S+ | A S−/NA S− |
| Tryptase | 0.007 | 0.005 | 0.005 | 0.005 | NS | NS |
| Histamine | NS | NS | NS | NS | NS | NS |
| EPX | 0.003 | 0.01 | 0.01 | 0.05 | 0.01 | NS |
| MPO | 0.01 | 0.01 | 0.01 | 0.01 | NS | NS |
| $LTC_4$ | 0.01 | NS | NS | NS | NS | NS |
| $PGD_2$ | 0.01 | NS | NS | NS | NS | NS |
| Neutrophils | NS | NS | 0.03 | 0.05 | 0.02 | NS |
| Eosinophils | 0.03 | NS | NS | NS | 0.04 | NS |

Statistical analysis by Mann-Whitney U test.

groups of patients they were enumerated in 33.3% to 56.5% (Table II). By Kruskal-Wallis test there was no significant difference between groups for any of the cell types studied. The percentage of eosinophils was significantly greater in the A S+ group than in the three other groups of patients (Fig. 1, Table III). Neutrophils were enumerated in 55.6% to 100% of patients with readable slides, and the greatest numbers were found in nonallergic individuals (Fig. 1, Table III). However, only eight patients had neutrophils over 25% of enumerated cells. Neutrophils were significantly increased in the NA S+ and NA S− groups, and these cells appeared to present a normal morphologic make up.

### Mediator levels in nasal lavage fluid

Levels of mediators and statistical analyses are given in Tables II and III and Fig. 2. By Kruskal-Wallis tests a significant difference occurred between groups for EPX ($p < 0.0001$) and $LTC_4/D_4$ ($p < 0.0001$).

Tryptase levels were undetectable in all the normal individuals but were detected in all but five patients. Levels ranged from undetectable to 1.9 μg/L. A significant increase occurred in tryptase levels in all four groups of patients, but no significant difference occurred between the groups. Histamine levels were detectable in all patients and subjects tested, and no significant difference occurred between groups.

EPX was detectable in four control subjects and most patients with chronic rhinitis ($p < 0.002$, Mann-Whitney U test). Moreover, although some overlap exists between groups of patients with chronic rhinitis, statistical differences exist between A S+ and A S− groups ($p < 0.05$, Mann-Whitney U test) and A S+ and NA S+ patients ($p < 0.01$, Mann-Whitney U test).

Myeloperoxidase levels were undetectable in control subjects and in 50% of patients. A significant difference was observed between control subjects and patients with rhinitis ($p < 0.005$, Mann-Whitney U test). However, no significant difference occurred between the four groups of patients.

$LTC_4/D_4$ levels were undetectable or low in the control group. They were significantly increased in the A S+ group in comparison with control subjects ($p < 0.01$, Mann-Whitney U test) and the A S− group ($p < 0.01$, Mann-Whitney U test). More than 70% of symptomatic patients (A S+ and NA S+) had $LTC_4/D_4$ levels over the highest level found in the control group. $PGD_2$ levels were low, under 10 ng/L in the control group and significantly higher in the A S+ group ($p < 0.01$, Mann-Whitney U test).

### Correlations between parameters

In patients with rhinitis a significant correlation was observed between $LTC_4/D_4$ and EPX ($r_s = 0.41$, $p < 0.005$, Spearman rank correlation). No other significant correlation was observed.

## DISCUSSION

This study examined nasal lavage cells and six inflammatory mediators released in nasal secretions of four groups of patients with perennial rhinitis and a control group. Patients with symptomatic allergic rhinitis had a significant increase of eosinophils in nasal secretions as well as an increase of EPX, $LTC_4/D_4$ and to a lesser extent $PGD_2$. Patients with nonallergic rhinitis, whether or not they were symptomatic, presented an increase in neutrophils and EPX. Tryptase, and neutrophil myeloperoxidase were increased in all rhinitis groups.

Nasal inflammation can be studied by different methods. Nasal biopsies directly identify inflammatory cells and mucosal damage,[31-35, 70] but they usually give little information on the activation state of the cells, cannot give quantitative results, and do not discern the possible heterogeneity of mucosal lesions.

| Symptomatic vs nonsymptomatic | | Allergic vs nonallergic |
|---|---|---|
| A S+/A S− | NA S+/NA S− | |
| NS | NS | NS |
| NS | NS | NS |
| 0.05 | NS | 0.008 |
| NS | NS | NS |
| 0.01 | NS | NS |
| NS | NS | NS |
| NS | NS | 0.03 |
| NS | NS | NS |

Hence, in this study we used nasal lavage because it indirectly identifies nasal inflammation by the enumeration of cells and measurement of mediators, provides information on a more extensive area of the nose, and is quantitative. However, some mediators may be degraded, and even if an increased level of a specific mediator is found in lavage fluid, the concentration of this mediator at the site of its action and whether it really participates in the reaction is unknown.[28] Even less is known about whether certain mediators and/or cytokines act in combination, increasing or decreasing the response of the others.[1] However, a mediator in nasal secretions may be considered as a marker of activity of a specific cell. This appears to be the case for EPX (eosinophils),[44, 45, 60] histamine (basophils and mast cells),[46] tryptase (mast cells),[47-49] and myeloperoxidase (neutrophils).[50] We did not concentrate the lavage fluid. Although this may have led to an increase in the detectability of certain mediators such as myeloperoxidase, concentration is not completely quantitative. The enumeration of cells may be improved by use of the Rhinoprobe (Rhino-Technics, San Diego, Calif.),[53] but this might modify in turn the release of mediators. Moreover, obtaining three lavages may also increase the yield of the cells. Finally, the analysis of inflammatory cells requires both the quantitation of the total cell number as well as the differential cell count. In the present article we only characterized the differential pattern, and the lack of clear discrimination between the different rhinitis groups may be related with the fact that we did not give cell counts.

The assessment of the clinical score of rhinitis was based on symptoms commonly occurring in rhinitis and on our experience of seasonal allergic rhinitis. In fact, as for many other allergic diseases there is no accepted symptomatic score that has been previously validated. Patients answered a questionnaire on the day of the nasal lavage, and there may be some biases

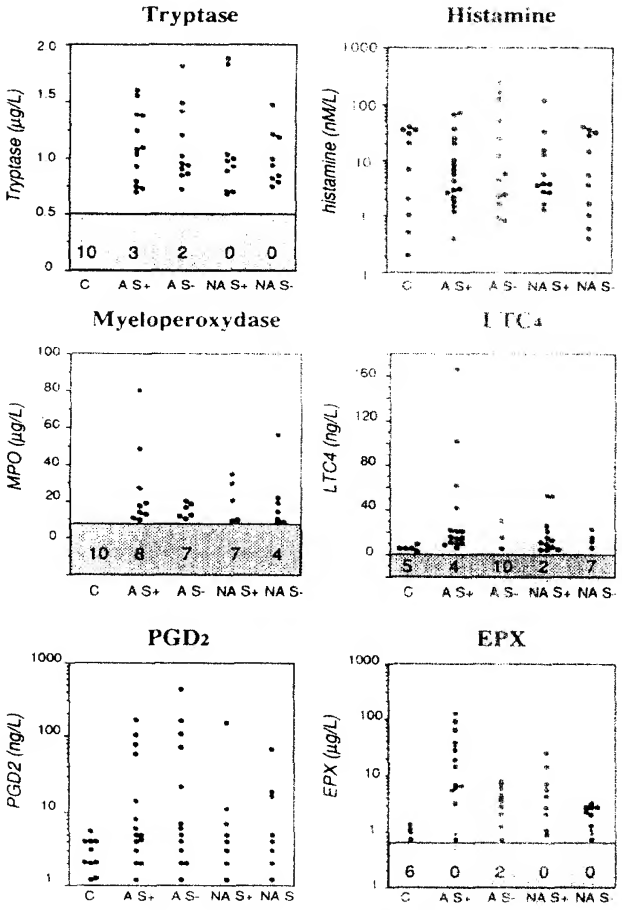


**FIG. 2.** Levels of mediators in nasal secretions. *C*, Control subjects. Numbers indicate patients with undetectable mediator.

for patients who were symptom-free (A S− and NA S−) in describing their symptoms. However, because we wanted to analyze the inflammatory events in all four groups of patients there was no other option. The discrimination between allergic and nonallergic patients was clear-cut, because all patients about whom we had questions were withdrawn from the study. As has already been reported patients with allergic rhinitis were significantly younger than those with nonallergic rhinitis. In the latter group, although anterior rhinorrhea was a common symptom, only a subset of patients had the so-called NARES,[36, 37] since eosinophils were found in approximately one third of these patients.

The original features of this paper are (1) the study of untreated, perfectly characterized patients with perennial rhinitis, (2) the study of symptomatic and symptom-free patients in an attempt to find out whether there might be a mediator that would differentiate these two groups of patients, (3) the use of

many mediators relevant to the allergic inflammation, and (4) the use of EPX as a marker of eosinophil activation.

One of the pitfalls that might be raised by this study is the lack of diagnosis of allergic patients. It is generally accepted that prick tests are less sensitive, less reproducible, but more specific than intradermal tests, thus the value of prick tests is limited by low potency extracts inducing false-negative results. With standardized extracts the prick test appears to be sensitive enough, and this method has been recommended even for research purposes. It is considered that prick test correlates better with symptoms, although in patients with a low sensitivity, intradermal skin tests may be the only positive test. All patients had a Phadiatop to confirm the results of skin prick tests. Moreover, in our area more than 95% of the patients with chronic allergic symptoms are sensitized to one or more allergen extracts that are in standardized form, and the Phadiatop is positive in more than 87% of patients allergic to perennial allergens (unpublished data) so that the possibility of missing an allergic patient is small. Thus overlap in nasal findings in the different groups was analyzed by Kruskal-Wallis test and might reflect in part some "low sensitivity" allergic patients included in the population with negative prick test outcomes. Using intradermal skin tests we might have identified some of the patients with negative prick tests. Another difficulty in the characterization of the patients is selection of asymptomatic patients. It is clear that asymptomatic inflammation may persist for some days after allergic or nonallergic triggers. This can be easily demonstrated in asthma with use of nonspecific hypersensitivity tests. Thus residual inflammatory changes in the nasal mucosa of individuals recently, but not currently, symptomatic may have led to overlaps in nasal findings in the symptomatic and asymptomatic groups.

The role of eosinophils in rhinitis has been demonstrated in seasonal and perennial rhinitis,* but although a time relationship appears to exist between the increase in eosinophils and levels of ECP or major basic protein and the development of symptoms of a late-phase reaction after allergen challenge, no individual relationship between these factors has been found.[16, 35] In the present study eosinophils were significantly increased in the A S+ group, confirming the importance of these cells in allergic perennial rhinitis. EPX was found to be of great value because most patients had detectable levels in contradistinction to normal individuals, and A S+ patients had the greatest levels. This study therefore confirms the role of eosinophils in allergic and nonallergic rhinitis. The measurement of EPX may be of greater value than the enumeration of eosinophils in the diagnosis of chronic rhinitis, but the number of patients is too low in the present study to make definite conclusions.

Histamine is inconstantly released during the immediate- and the late-phase reactions after allergen challenge.[2, 8-10, 15, 20, 26] It does not always correlate with the occurrence of symptoms,[8, 9] and the levels of pharmacologically active histamine in nasal secretions may be very high in asymptomatic patients before any challenge and may be increased during viral infections.[8, 9, 30] During allergen challenge, the release of histamine does not appear to be significantly correlated with those of tryptase.[15] During the pollen season, levels of histamine in nasal secretions were found to be similar than before in some studies,[31] and one study of perennial rhinitis found that histamine levels were not increased.[42] In the present study we did not find elevated levels of histamine in nasal secretions of any of the groups of patients, whereas tryptase was elevated in all these four groups. This discrepancy cannot be explained by the method of titration used. The increased levels of tryptase in the four groups of patients suggest that mast cells are activated both in allergic and nonallergic rhinitis. This finding appears to be similar to the situation observed in nonallergic asthma in which tryptase levels are increased in bronchoalveolar lavage fluid. Thus although mast cells appear to be involved in chronic rhinitis, the titration of mast cell–derived mediators released in nasal secretions might not be totally adequate to explain the mechanisms of the nasal allergic reaction.

The role of neutrophils in allergic rhinitis remains to be clarified. These cells are usually increased when lavages are carried out 3 to 8 hours after challenge, but they are present both in patients with and without a late-phase reaction, and the presence of neutrophils is not discriminative.[5, 6, 22-24] These cells are also often observed in seasonal allergic rhinitis and in noninfectious perennial rhinitis,[27, 34, 72] and in the present study neutrophils were mainly increased in the nonallergic group (NA S+ and NA S−). Myeloperoxidase is released by activated neutrophils and was found to be increased in many patients of all four groups without any difference between the groups. Taken together, neutrophils may be involved in perennial rhinitis, but the significance of these findings needs clarification.

Other vasoactive mediators including $PGD_2$ and sulfidopeptide leukotrienes are also released during a nasal challenge.[3, 4, 7-12, 23] Although they do not possess all the vasoactive activities of histamine, particularly the sensitory nerve stimulation, they appear critical

*References 18, 28, 29, 34, 36, 37, 40, 70.

to the allergic reaction, and $PGD_2$ was found to correlate better than histamine with the occurrence of symptoms during nasal challenge.[7] These two mediators were increased in the A S+ group only when compared with normal subjects, and $LTC_4/D_4$ may be of importance because this was the second mediator of the study to be increased in the symptomatic group. This finding accords with data observed in seasonal rhinitis where sulfidopeptide leukotrienes were increased during the pollen season.[26, 30] Sulfidopeptide leukotrienes are synthetized by eosinophils,[72, 73] and these increased levels may be related with eosinophil activation as suggested in the present study by the significant correlation between $LTC_4/D_4$ and EPX levels in patients with rhinitis.

In conclusion, this study suggests a prominant role for the eosinophil in the inflammatory reactions occurring in patients with chronic rhinitis. The titration of EPX and eventually $LTC_4/D_4$ appear to be valuable tools in the characterization of such patients.

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# Allergenicity of peanut and soybean extracts altered by chemical or thermal denaturation in patients with atopic dermatitis and positive food challenges

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*Peanuts and soybeans are two of the six most common foods to cause food hypersensitivity reactions in children. We used the serum of 10 patients with atopic dermatitis and positive double-blind, placebo-controlled, food challenges to peanut and two patients with atopic dermatitis and positive double-blind, placebo-controlled, food challenges to soybean to investigate the change in IgE-specific and IgG-specific binding to these proteins altered by either chemical or thermal denaturation. We used IgE- and IgG-specific ELISA-inhibition analyses to compare these effects on the crude peanut and crude soy extracts, as well as on the major allergenic fractions of both proteins. Heating the soy proteins at various temperatures and time intervals did not significantly change the IgE- or IgG-specific binding of the soy positive pooled serum. When the peanut proteins were subjected to similar heating experiments, the IgE- and IgG-specific binding did not change. When these same proteins were treated with enzymes in the immobilized digestive enzyme assay system used to mimic human digestion, the binding of IgE to the crude peanut and crude soy extracts was reduced; 100-fold for peanut and 10-fold for soybean. Therefore it appears that thermal denaturation of peanut and soybean protein extracts does not enhance or reduce IgE- and IgG-specific binding activity. Chemical denaturation appears to minimally reduce the binding of these proteins. (J ALLERGY CLIN IMMUNOL 1992;90:889-97.)*

***Key words:*** *Peanut allergens, soybeam allergens, food hypersensitivity, atopic dermatitis*

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Multiple allergens have been identified in the past several years that stimulate IgE-mediated disease in humans. The identification and purification of these allergens is essential for further studies to understand and characterize the immune response to these antigens.[1] Structural studies of these allergens is also critical to the understanding of the IgE-mediated response.[2] Several inhaled allergens have been characterized from a wide variety of sources, including dust mites, pollens, animal danders, insects, and fungi. Only recently have food allergens been studied with